Myocardial lesions induced by prolonged alcohol feeding in rhesus monkeys

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AUTHORS' SYNOPSIS
To elucidate the effects of chronic alcohol ingestion in monkeys a synthetic, adequately balanced, fluid diet providing 40% of total calories from ethanol was gavaged through a stomach tube daily over a period of three months. Clinical, biochemical, radiosotope, and histopathological studies were performed at the beginning and end of the experiment. It was observed that chronic alcohol feeding at this dose level caused marked accumulation of triglycerides, cholesterol, and phospholipids in the serum and the liver. In the heart triglycerides and cholesterol ester were increased. Incorporation studies showed increased synthesis of triglycerides in the heart muscle and liver. Histologically the heart showed fatty change of the myocardium and evidence of focal myocytolysis, atrophy of muscle bundles, and early fibrosis. The liver showed generalized fatty change but no cirrhosis.

Ever since Evans (1961) described the syndrome of alcoholic cardiomyopathy in man, several clinical reports (Wendt et al, 1965; Burch and De Pasquale, 1968) and experimental studies in small laboratory animals (Bishop et al, 1967; Maines and Aldinger, 1967; Marciniak et al, 1968) have been published. So far the pathological and biochemical lesions produced in experimental animals by alcohol feeding only partially simulated the myocardial pathology seen in chronic alcoholic patients. Experimental studies on rats have revealed that the damage of the heart muscle is brought about through deposition of lipids and several ultrastructural alterations in the myocardial muscle occur (Bishop et al, 1967; Hall and Rowland, 1970). It was thought that the rhesus monkey, a species phylogenetically, metabolically, and behaviourally more akin to man may prove a better experimental animal for the study of the deleterious effects of alcohol on the heart. In this experiment high doses of alcohol have been fed to rhesus monkeys for a period of 3 months and structural and biochemical changes in the myocardium have been studied.

Material and methods
Adult healthy male rhesus monkeys, body weight 4–6 kg, were first acclimatized in the laboratory for a period of 2 months. They were subjected to a clinical, haematological, and pathological examination to exclude any obvious disease. They were divided into two groups; the experimental group (I) containing 14 animals and the control group (II) containing 12 animals. They were fed for 3 months on a synthetic liquid diet adequate in all proximate principles, vitamins, and minerals (Table 1). The diet was given twice daily at 11 am and 4 pm through a stomach tube.

In the experimental group ethyl alcohol partially replaced the carbohydrates to provide 40% of total calories. Before starting the experiment the animals were fasted overnight and blood was collected from the femoral vein and lipids extracted (Folch
Alcohol fed ether: diethyl ether: acetic acid chromatography on Silica gel factors were added to using a solvent mixture containing petroleum phospholipids (Bartlett, triglycerides (van Handel and Zilversmit,)

Adequate quantities of vitamins, minerals, and lipotropic factors were added to the diet.

et al, 1957). Serum cholesterol (Zak, 1957), triglycerides (van Handel and Zilversmit, 1957), phospholipids (Bartlett, 1959), and their fractions were estimated after separation by thin layer chromatography on Silica gel G (E. Merck Co) using a solvent mixture containing petroleum ether:diethyl ether:acetic acid (90:10:1 by volume). Total serum protein and lipoproteins were estimated by paper electrophoresis. These investigations were repeated at the end of the experiment. A number of studies were undertaken using palmitate 1-14C to determine the rate of synthesis of triglycerides in the heart muscle. Before sacrifice the animals were given palmitate 1-14C (25 μc/kg body weight, iv); they were sacrificed after 30 min. The plasma, liver, and heart were studied for the incorporation of the radio isotope into triglycerides.

The animals were sacrificed by exsanguination and a complete necropsy performed. The thoracic cavity was opened and after splitting the pericardium the heart was examined in situ. Then the heart was removed by dismembering it from the great vessels at the base, the weight was recorded. The right and left ventricles were slit open and the internal surface examined. The abdominal cavity was explored for any gross pathological change and the pancreas, kidneys, and spleen were removed. The weight of the liver was recorded. The aorta and lungs were also dissected out. Tissues were fixed in buffered 4% formaldehyde solution and blocks were processed and embedded in paraffin wax. Thin sections were prepared and stained with haematoxylin and eosin and haematoxylin basic fuchsin picric acid (Lie et al, 1971) for detection of early myocardial damage. Mallory's PTAH and Masson's trichrome stain were employed to detect changes in the muscle and the fibrous tissue stroma of the myocardium. Fresh tissue sections were cut in a cryostat and stained with Sudan IV to demonstrate tissue lipidosis. The sections were examined separately by two independent observers who had no knowledge to which groups the sections belonged. This was done intentionally to avoid a biased opinion.

### Results

It was noted that after each feed of the alcohol diet the animals became drowsy and that they clung to the bars of the cage. This state lasted for about 2–3 hr. Both groups of animals showed a gradual increase in body weight. The average weight of animals in the control group rose from 4.14 kg to 5.07 kg while alcohol-fed animals weighed 4.64 kg initially which rose to 5.33 kg at the end of the 3 months. On clinical examination they did not show any major abnormality. The pulse rate at the start of experiment was 242 ± 15 per minute and after 3 months of alcohol feeding it was 204 ± 28. Similarly, the systolic blood pressure was 116 ± 8.1 mm Hg initially and 120 ± 3.5 mm Hg at the end of 3 months. Initial diastolic pressure was 77 ± 3.9 mm Hg and 78 ± 2.6 mm Hg after 3 months. Clinical auscultation of the heart sound showed no alteration. The electrocardiograms done serially were also unaltered. Chest radiology was however, not done. The animals' fur was shiny. There were no gastrointestinal disturbances. Routine haematological examination revealed no anaemia nor any other abnormality.

### Pathological lesions

The gross appearance of the heart was flabby. The heart weight/body weight of 4.34 ± 0.19 showed an increase over the control value (3.65 ± 0.13). Externally the heart showed no lesion but the epicardial fat appeared to be increased in the alcohol-fed animals. The ventricular chambers and endocardial surface also appeared normal. The liver appeared to be enlarged, flabby, and presented a diffuse nutmeg appearance in the alcohol-fed animals (liver weight/body weight was 21.95 ± 0.31 compared to 20.22 ± 0.74 in controls). The cut surface showed mottling and appeared to be congested. The pancreas, kidneys, spleen, and lungs showed no gross lesions.

In alcohol-fed animals microscopic examination of the heart at the base and the middle zone showed evidence of fatty change in the myocardium. The fat vacuoles appeared to present a signet ring appearance in many muscle fibres; this was mostly due to neutral fat (Fig. 1) as confirmed by Sudan IV staining. The Schultz histochemical test for detection of cholesterol

### TABLE I

**Composition of diet expressed in percentage calories**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sucrose</th>
<th>Starch</th>
<th>Casein</th>
<th>Vegetable oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>30</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Alcohol fed</td>
<td>0</td>
<td>20</td>
<td>18</td>
<td>22</td>
</tr>
</tbody>
</table>

* Adequate quantities of vitamins, minerals, and lipotropic factors were added to the diet.
FIG. 1  Section of the heart of a monkey fed alcohol for 3 months. There is diffuse fatty change of the myocardium and the muscle nuclei show variable changes. Some fatty inclusions have a signet ring appearance. (H & E, × 400.)

FIG. 2  Section of the heart of a monkey fed alcohol for 3 months. There is evidence of patchy myocardial degeneration (dark staining areas). There is no evidence of inflammatory cellular reaction. (Haematoxylin-basic fuchsin-picric acid, × 100.)

FIG. 3  Section of the heart of a monkey fed alcohol for 3 months, showing fragmentation of muscle fibres. There is separation of muscle bundles by increased intermystial stroma. (H & E, × 40.)
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**FIG. 4** High power view of the section in Fig. 3 showing loss of muscle striations and various sizes of the muscle bundles. The intercalated discs are not clearly shown. (H & E, × 100.)

**FIG. 5** Section of the heart of a monkey fed alcohol for 3 months. There is evidence of early fibrotic changes in the myocardium. Apart from this, the muscle bundles show atrophic changes. (H & E, × 100.)

**FIG. 6** Section of the liver of a monkey fed alcohol for 3 months. There is diffuse fatty infiltration of the hepatic cell but no cirrhosis. (H & E, × 100.)
ester was also positive in a number of cases. To detect early myocardial degeneration HBFP staining (Lie et al., 1971) was performed routinely and demonstrated patchy lesions in the myocardium which were distributed irregularly in both the ventricular walls and in the interventricular septum (Fig. 2). This histochemical staining method was useful in differentiating the degenerative fibres from normal ones. The other features which were noted in the myocardium were gross variation in the size of the muscle fibres, fragmentation, and loss of striations with a variable degree of nuclear atypism (Figs. 3 and 4). Inflammatory reaction in the myocardium was not a prominent feature. In some animals early fibrosis of the myocardial muscle was seen and there were areas where muscle atrophy was prominent (Fig. 5). The coronary arteries generally appeared normal. The appearance of the endocardium and pericardium was unremarkable.

In the control animals the pericardium, myocardium, and endocardium did not present any pathological lesion. Microscopic examination of the liver in alcohol-fed animals showed diffuse marked hepatic lipidosis (Fig. 6). There was no evidence of cirrhosis. The pancreas, kidneys, spleen, aorta, and lungs did not present any lesion.

**Biochemical changes**

The results of plasma and tissue lipids in controls and alcohol-fed monkeys are presented in Table 2. It can be seen that the alcohol diet resulted in an increase in plasma of triglycerides, cholesterol, and phospholipids by about 34%, 23%, and 49%, respectively. The increase in cholesterol was due to a rise in both free and esterified cholesterol. The data on tissue lipids reveal that in liver there was a five-fold rise of triglycerides and a significant increase in cholesterol (50%) and phospholipids (33%) in the liver. As in plasma, both cholesterol and cholesterol ester contributed to the increased sterol content of liver. In the heart (Table 2) there was a three-fold rise of triglycerides with a

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Plasma</th>
<th>Liver</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Alcohol</td>
<td>Control</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>58.05 ± 3.33</td>
<td>78.50 ± 5.79</td>
<td>3.41 ± 0.43</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>148.34 ± 6.63</td>
<td>182.98 ± 6.86</td>
<td>3.86 ± 0.27</td>
</tr>
<tr>
<td>Ester</td>
<td>114.68 ± 5.84</td>
<td>130.65 ± 5.52</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>Free</td>
<td>33.60 ± 1.52</td>
<td>52.33 ± 2.27</td>
<td>1.35 ± 0.18</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>157.65 ± 6.96</td>
<td>235.76 ± 18.94</td>
<td>21.91 ± 1.04</td>
</tr>
</tbody>
</table>

* The difference between the alcohol-fed animals and the control was not significant.

<table>
<thead>
<tr>
<th>CPM (100 g of tissue)</th>
<th>Liver</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Alcohol</td>
<td>Control</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>6,900 ± 230</td>
<td>10,100 ± 270</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>19,700 ± 310</td>
<td>36,100 ± 560</td>
</tr>
</tbody>
</table>

Palmitate 1-C¹⁴ (25 μci/kg body weight) was injected intravenously and the animals sacrificed after 30 min. Free fatty acids and triglycerides were separated by thin layer chromatography.
significant increase in cholesterol ester (P < 0.001), total cholesterol being unaffected. No significant change was seen in the total and differential phospholipids in the heart. The extent of incorporation of palmitate $^{1-14}$C into free fatty acids and triglycerides of plasma, liver, and heart is shown in Table 3. It was found that there was increased incorporation of palmitate $^{1-14}$C into free fatty acids and triglycerides of liver and heart and plasma following alcohol ingestion.

**Discussion**

The present experiment in rhesus monkeys has shown that prolonged feeding of high doses of alcohol produced marked increase of lipids in the plasma and tissues (liver and heart). The chief component of the lipids was triglycerides which were deposited in the heart muscle and liver cells causing distortion of their structure. Cholesterol ester was also increased in the heart muscle whereas in the liver there was increase of both free and ester cholesterol and phospholipids. In earlier reports on dogs and rats (Lieber et al., 1966; Marciniak et al., 1968), it was shown that prolonged ethanol administration caused a marked rise of triglycerides in the plasma and heart. However, these previous studies did not elucidate its mechanism of accumulation nor did they elaborate the various structural changes produced in the hearts of alcohol-fed animals. The general belief is that alcohol ingestion diminishes the utilization of fatty acids by heart muscle which ultimately leads to their accumulation. Studies *in vitro* have shown that following acute alcohol infusion in rabbits there is increased incorporation of palmitate $^{1-14}$C into triglycerides of heart muscle slices (Kikuchi and Kako, 1970).

The present experiment on monkeys utilizing *in-vivo* techniques has supported the earlier findings and has further proved that chronic ingestion of alcohol leads to marked deposits of triglycerides in the heart muscle through increased synthesis of this lipid locally. The lipid deposition in the liver in these animals is also due to increased synthesis.

The chief histological feature noted in the heart and the liver was diffuse accumulation of neutral fat in the parenchymal cells. Histochemical studies also showed some increase of cholesterol ester. In addition to this the myocardium showed patchy areas undergoing early degeneration as revealed by a positive reaction to HBFP staining. This feature was only seen in alcohol fed animals.

Besides this, atrophy of the myocardial muscle cells with fragmentation of the muscle fibres and thin fibrous bands traversing these areas were seen. In none of these sections of the heart was any significant inflammatory reaction noted. This provided some proof that the changes noted in the myocardium in alcohol-fed animals were mainly the result of metabolic abnormalities and that they were not produced by an infective process. It, therefore becomes evident that the heart muscle is susceptible to alcoholic injury, since no other factor such as dietary inadequacy or vitamin deficiency were co-existent. In earlier electron microscopy studies (Hibbs et al., 1965; Bishop et al., 1967) ultrastructural changes in the heart muscle have been demonstrated both in human beings and in rats following chronic alcoholism. It has been noted that there is myofibrillar disorganization, increase of sarcoplasmic reticulum, mitochondrial swelling, and rupture and fatty inclusions in the cells. However, so far only minimal changes have been noted in the heart using light microscopy. In view of this the present findings of both structural and biochemical changes in the heart muscle in chronic alcohol fed monkeys assumes significance.

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**References**


